REMARKS

Claims 1 – 7, 13, 14, 17, 18, 23 – 29, 31 – 45, 50, 52, 54 and 56 are pending in the application. Claims 2, 3, 8 – 12 and 15 – 56 have been cancelled. Claims 1, 5 and 6 have been amended. No new matter has been added by virtue of these amendments; support therefore can be found in throughout the specification and original claims of the application. Specifically, support for the amendment to claim 1 to include the selection marker gene can be found in the specification on page 16, line 33 through page 17. Support for the amendment to claim 1 to include various insulator sequences can be found on page 23, lines 2 – 6 and page 53, lines 4 – 5.

Any cancellation of the claims should in no way be construed as acquiescence to any of the Examiner's rejections and was done solely to expedite the prosecution of the application. Applicant reserves the right to pursue the claims as originally filed in this or a separate application(s).

Priority

The Examiner acknowledges Applicants claim for domestic priority under 35 USC 119 (a) – (d).

Information Disclosure Statement

The Examiner indicates that the information disclosure statement filed March 3, 2005 fails to comply with 37 CFR 1.98(a)(3). The Examiner argues that "in particular, English translations of citations FC, GA and HB have not been provided." (Office Action, p,.4).

Applicants submit herein an information disclosure statement in compliance with 37 CFR 1.98(a)(3) for the Examiner's consideration.

Claim Objections

The Examiner has objected to claim 3 as being drawn to non-elected subject matter. Claim 3 has been cancelled.

Applicants respectfully request that the objection be withdrawn.

Rejection of Claims 1, 3 – 7 and 13 - 14 Under 35 USC 112, first paragraph

Written Description

The Examiner has rejected claims 1, 3 – 7 and 13 - 14 under 35 USC 112, first paragraph for failing to comply with the written description requirement. The Examiner argues that claims 1, 3 – 7 and 13 – 14 contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the invention as claimed. Applicants respectfully disagree.

The Examiner argues that,

> (t)he claimed invention is directed to a method of making a mammalian artificial chromosome, wherein the artificial chromosome comprises an insulator sequence (and) at issue is the lack of adequate description of an insulator sequence. (Office Action, p.5).

The Examiner further alleges that the specification "does not disclose any identifying structural characteristic as to how an artisan would *a priori* know that a given nucleic acid sequence is an insulator sequence." (Office Action, p.7). The Examiner argues that the one insulator sequence that is disclosed, the human beta globin locus control region (LCR) comprising HS1 – HS5, is not representative of the genus because the genus of insulator sequences is highly variant. Applicants disagree, however in the interest of advancing prosecution have amended the claims.

The amended claims recite certain insulator sequences selected from human beta-globin HS1 to 5, chicken beta globin HS4, Drosophila gypsy retrotransposon, sea urchin 5' flanking region of arylsulfatase, blocking element α/ γ;of human T cell receptor α/ γ, and repeat organizer of Xenopus 40S ribosomal RNA gene.

First, the specification provides clear teaching regarding the function and effects of insulator sequences:

> the insulator sequence is a base sequence characterized by exhibiting an enhancer blocking effect (expressions of neighboring genes are not affected by each other) or a chromosome boundary effect (a region assuring the gene expression and a region suppressing the gene expression are separated with each other). It is expected that the use of the insulator sequence promotes the expression of a target

> gene contained by a mammalian artificial chromosome. ([0160]).

Moreover, the specification provides support for this claimed group of insulator sequences. Applicants direct the Examiner to paragraph [0160] of the specification, which points out that:

> human B globin HS1 to 5, chicken β-globin HS4, Drosophila gypsy retrotransposon, sea urchin 5' flanking region of arylsulfatase, blocking element α/d of human T-cell receptor α/d, repeat organizer of Xenopus 40S ribosomal RNA gene, and the like, have been **known as insulator sequence**. (emphasis added).

Accordingly, Applicants request that the rejection be withdrawn.

Enablement

The Examiner has rejected claims 1, 3 – 7 and 13 - 14 Under 35 USC 112, first paragraph for failing to comply with the enablement requirement. The Examiner argues that the specification, while enabling for a method of making a mammalian artificial chromosome as claimed comprising a sequence of interest and a beta-globin locus control region insulator sequence," does not reasonably provide enablement for a genus of insulator sequences." (Office Action, p.9). Applicants respectfully disagree.

The amount of experimentation required to practice the invention as claimed is not undue. As stated in the MPEP at § 2164.01(a):

There are many factors to be considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is "undue." These factors include, but are not limited to: (A) The breadth of the claims; (B) The nature of the invention; (C) The state of the prior art; (D) The level of one of ordinary skill; (E) The level of predictability in the art; (F) The amount of direction provided by the inventor; (G) The existence of working examples; and (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure.

The amended claims recite certain insulator sequences selected from human beta-globin HS1 to 5, chicken beta globin HS4, Drosophila gypsy retrotransposon, sea

urchin 5' flanking region of arylsulfatase, blocking element α/ γ;of human T cell receptor α/ γ, and repeat organizer of Xenopus 40S ribosomal RNA gene.

Applicants submit that, in view of the instant amendments, the claims are fully enabled.

Applicants have provided ample working examples in the instant specification. Applicants have provided Examples 1 – 15, which teach the construction of alphoid BAC (example 1), generation of HAC (example 2), DNA structure of HACs (example 4), GCH1 expression from HAC (example 6). In example 15, beginning at paragraph [0266], Applicants teach the construction of mammalian artificial chromosome having a gene insertion site.

The specification teaches the function of insulator sequences at paragraphs [0160] – [0162], and gives examples of insulator sequences. Moreover, insulator sequences as described in the specification were known in the art at or prior to the time of filing. For example, Wei et al (Mol Cell Biol. 2001 Nov;21(22):7714-20) describe the gypsy insulator as a promoter-specific transcriptional stimulator and Gause et al. (Mol Cell Biol. 2001 Jul;21(14):4807-17) teach insulation of enhancer-promoter communication by a gypsy transposon insert in a Drosophila gene. Burgess-Buesse et al. (Proc Natl Acad Sci U S A. 2002 Dec 10;99 Suppl 4:16433-7) describe an insulator at the 5' end of the chicken beta-globin locus that marks a boundary between an open chromatin domain and a region of constitutively condensed chromatin. Thus, the claimed insulator sequences were known in the art to enable one of skill in the art to perform the invention as claimed.

Accordingly, Applicants submit that the invention is enabled as claimed.

Rejection of Claims 1, 3 – 7 and 13 - 14 Under 35 USC 112, second paragraph

The Examiner has rejected claims 1, 3 – 7 and 13 – 14 for alleged indefiniteness. Applicants respectfully traverse the rejection.

The Examiner argues that "with respect to claim 1 (and dependent claims), the claims are vague in that no step(s) in the claimed method refers back to or recapitulates the preamble of the claim." (Office Action, p.12). Applicants have amended the claims and respectfully request that the rejection be withdrawn.

The Examiner argues that "with respect to claim 1, the grammatical structure of the first step is unclear because a plurality of elements, delimited by only a conjunction

"and", are to be introduced into a mammalian host cell." (Office Action, p.12). Applicants have amended the claims and respectfully request that the rejection be withdrawn.

The Examiner argues that "with respect to claim 1 (and dependent claims), the claims are incomplete for omitting essential steps, such omission amounting to a gap between the steps." (Office Action, p.12). The Examiner indicates that "the omitted steps are: the means by which the cell containing the mammalian artificial chromosome is to be selected from the transformed cells." (Office Action, p.12). Applicants have amended the claims and respectfully request that the rejection be withdrawn.

The Examiner argues that "with respect to claim 3, [the claim] recite the limitation 'the selection marker gene' in reference to the selection step of claim 1 (and) there is insufficient antecedent basis for this limitation in the claim." (Office Action, p.13). Applicants have amended the claims to recite proper antecedent basis and respectfully request that the rejection be withdrawn.

The Examiner argues that "with respect to claims 5 – 6, the claims recite a sequence derived from a human chromosome alpha satellite region...of a human chromosome 21 (and) the claims are vague and indefinite in that the metes and bounds of the term 'derived from' are unclear." The Examiner indicates that "it would be remedial to amend the claim language to use the term 'obtained from'." (Office Action, p.13). Applicants have amended the claims according to the Examiner's suggestions and respectfully request that the rejection be withdrawn.

The Examiner argues that "with respect to claim 1 and 13, the claims are incomplete for omitting essential elements, such omission amounting to a gap between the steps." (Office Action, p.12). The Examiner argues that the omitted elements are a loxP site present in the first vector, and the instantly claimed method does not recite the first vector to comprise a loxP site necessary for recombination cloning, and the claims do not recite the presence of Cre recombinase that acts upon the loxP sites to mediate recombination. Claim 1 as amended recites an insertion sequence for specifically inserting a sequence of interest. Claim 13 recites that the insertion sequence is a loxP site, a FRT site. Accordingly, there are no omitted elements accounting for a gap between the steps. Applicants respectfully request that the rejection be withdrawn.

Rejection of Claims 1, 3 – 6 and 13 Under 35 USC 103(a)

The Examiner has rejected claims 1, 3 – 6 and 13 under 35 U.S.C. § 103(a) as being unpatentable over Mejia et al. (Genomics 70(2):165-170, 2000), in further view of Waye et al. (Mol and Cell Biol 6(9):3156 – 3165, 1986), Ikeno et al. (Human Mol. gen 3(8):1245 – 1257, 1994) and Perkins et al. (US 2003/0119104). Applicants respectfully traverse the rejection.

The Examiner argues that the Mejia reference teaches,

> a method of making mammalian artificial chromosomes, the method comprising a step of introducing into a prokaryotic host cell a first vector...and a second vector...selecting the transformed cells and selecting a cell containing a mammalian artificial chromosome from the selected transformed cells. (Office Action, p.15).

The Examiner argues that the Mejia reference teaches that, "the first vector comprises a selection marker gene...(t)he mammalian centromere sequence comprises 220kb of alpha satellite DNA from human chromosome 17 centromere (and) (t)he insertion sequence is a loxP recombination site." (Office Action, p.15). The Examiner admits that the Mejia reference does "not teach the method being performed in mammalian cells, nor that the vector comprises an insulator sequence." (Office Action, p.15).

The instant invention is based on a novel production method of a mammalian artificial chromosome. The instant invention, unlike Mejia, produces a HAC that has the **capability of insertion** of a transgene of interest. The mammalian artificial chromosome of the instant invention has an insulator sequence **for the purpose of promoting the expression of a gene to be introduced later**, and it was found by the inventors that, surprisingly, the efficiency of gene transfer into the mammalian artificial chromosome was enhanced. Applicants direct the Examiner to the specification, where this is described at paragraphs [0009] and [0160]. In contrast, human artificial chromosomes (HACs) that are heretofore known in the art have the capacity to accommodate a large transgene, but are **generated *de novo* from a precursor construct** with both the transgene and an alphoid array.

The Mejia reference fails to teach or suggest all the elements of the instant invention. Specifically, the Mejia reference does not teach a production method of a mammalian artificial chromosome where the method comprises a first step of

introducing a first vector being circular in form and comprising a mammalian centromere sequence, and introducing a second vector being circular in form and comprising an insertion sequence for specifically inserting a sequence of interest and an insulator sequence into a mammalian host cell. The Mejia reference teaches a method of *de novo* construction of a human artificial chromosome comprising a transgene of interest (e.g. HPRT). Figure 1 on page 167 teaches the HAC construct taught by Mejia, that includes the genomic insert (the HPRT transgene) from the beginning. Figure 1 is " a circular map showing the large-scale structure of the 404-kb BAC lox.P sites (♦) mark the boundaries of the DNA components assembled by Cre-mediated recombination, namely the alpha satellite BAC **and the HPRT genomic insert**." (emphasis added).

The method of Mejia uses cre-lox recombination system **to construct a precursor** of HAC using recombination in ***E.coli***. The HAC constructed by the method as taught by Mejia has the genomic insert (i.e. HPRT) as a transgene **from the beginning**. Mejia does not teach or suggest a production method of a mammalian artificial chromosome where the method comprises a first step of introducing a first vector being circular in form and comprising a mammalian centromere sequence, and introducing a second vector being circular in form and comprising an insertion sequence for specifically inserting a sequence of interest and an insulator sequence into a mammalian host cell as instantly claimed.

None of the secondary references cure the defects of the Mejia reference. Combined, the Waye, Ikeno and Perkins references do not cure the flaws of the Mejia reference, nor do they teach or suggest a production method of a mammalian artificial chromosome as instantly claimed.

The Examiner has rejected claims 1 and 7 under 35 U.S.C. § 103(a) as being unpatentable over Mejia et al. (Genomics 70(2):165-170, 2000), in further view of Waye et al. (Mol and Cell Biol 6(9):3156 – 3165, 1986), Ikeno et al. (Human Mol. gen 3(8):1245 – 1257, 1994) and Perkins et al. (US 2003/0119104) as applied to the claims above, and further in view of Bokkelen et al. (US 5,695,967). Applicants respectfully traverse the rejection.

As described above, the Mejia reference fails to teach or suggest all the elements of the instant invention. Specifically, the Mejia reference does not teach the

claimed production method of a mammalian artificial chromosome where the method comprises a first step of introducing a first vector being circular in form and comprising a mammalian centromere sequence, and introducing a second vector being circular in form and comprising an insertion sequence for specifically inserting a sequence of interest and an insulator sequence into a mammalian host cell. The Mejia reference teaches human artificial chromosomes (HAC) that has the capacity to accommodate a large transgene, but is **generated *de novo* from a precursor construct** and already contains the transgene.

None of the secondary references cure the defects of the Mejia reference. Combined, the Waye, Ikeno, Perkins and Bokkelen references do not cure the flaws of the Mejia reference, nor do they teach or suggest a production method of a mammalian artificial chromosome as instantly claimed.

The Examiner has rejected claims 1 and 7 under 35 U.S.C. § 103(a) as being unpatentable over Mejia et al. (Genomics 70(2):165-170, 2000), in further view of Waye et al. (Mol and Cell Biol 6(9):3156 – 3165, 1986), Ikeno et al. (Human Mol. gen 3(8):1245 – 1257, 1994), Perkins et al. (US 2003/0119104) and Bokkelen, as applied to the claims above, and further in view of Cooke et al. (WO 00/18941).

As described above, the Mejia reference fails to teach or suggest all the elements of the instant invention. Specifically, the Mejia reference does not teach a production method of a mammalian artificial chromosome where the method comprises a first step of introducing a first vector being circular in form and comprising a mammalian centromere sequence, and introducing a second vector being circular in form and comprising an insertion sequence for specifically inserting a sequence of interest and an insulator sequence into a mammalian host cell. The Mejia reference teaches human artificial chromosomes (HAC) that has the capacity to accommodate a large transgene, but is **generated *de novo* from a precursor construct** and already contains the transgene.

None of the secondary references cure the defects of the Mejia reference. Combined, the Waye, Ikeno, Perkins, Bookelen and Cooke references do not cure the flaws of the Mejia reference, nor do they teach or suggest a production method of a mammalian artificial chromosome as instantly claimed.

Therefore, the teachings of the cited art, when combined, do not logically result in the claimed invention.

Accordingly, Applicants respectfully request that the rejection be withdrawn.

CONCLUSION

Early consideration and allowance of the application are earnestly solicited.

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Respectfully submitted,

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